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MINIMISING CALIBRATION PROBLEMS OF *IN VIVO* GLUCOSE SENSORS

FIELD OF INVENTION

Implanted or semi-implanted glucose sensors for monitoring of blood glucose in the
5   regulation of e.g. diabetes mellitus are well known. However, it is a significant problem
that the sensors presently available do not function adequately *in vivo* over a sufficient
time period in spite of the fact that the sensors function well *in vitro*. Hence, for state of
the art sensors it is necessary to calibrate the sensors a number of times e.g. at least four
times daily because the sensitivity of the sensor changes over time. Although a number of
10 reasons for this problem have been suggested, none of the proposed solutions have been
able to solve the problem and the reason for the sensitivity problem is still unknown.

The present invention relates to the finding that by using a glucose sensor with an outer
membrane comprising catalase and/or other reactive oxygen species scavengers, in order
15 to secure that reactive oxygen species do not diffuse out of the sensor to the surroundings,
the calibration problems are significantly reduced. It is important that the reactive oxygen
species is reduced to a concentration much lower than the concentration where it will
exert cytotoxic effects.

20 The present invention thus relates to a method of improving the performance of a ROS
producing glucose sensor, said method comprising providing the glucose sensor with a
ROS removing compartment capable of reducing the diffusion of ROS out of the glucose
sensor to a level at which biointerference is abolished or substantially reduced. The
invention further relates to use of a ROS removing compartment in a ROS producing
25 glucose, a ROS producing glucose sensor comprising a ROS removing compartment, and to
the use of such a sensor in a human.

BACKGROUND OF THE INVENTION

30 In some way biosensor implants represent an extreme variant of (xeno)transplantation
and a lot of relevant models of tissue interactions and relevant experimental data can be
drawn from basic immunological studies.

Immunology has been defined as the science of self-nonself discrimination. Slightly altered
35 conformation of the major plasma/lymph proteins at the surface of the implant may be an
initial trigger of immunological responses.

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Nonself does not necessary trigger a strong response. A "danger" signal is often also required. The danger signal may simply result from mechanical disruption of a few cells or capillary vessels and the release of cell membrane or tissue fragments. Besides

5   immunology, the literature related to wound healing and tissue repair is therefore also very relevant.

On a time scale the basic tissue interactions with an implant (or transplant) are:

10   Immediately/ short term (seconds to hours)
     Disruption of cells, release of cell fragments "danger" signals (=> clot formation, coagulation factors, complement factors, mobilisation of different inflammatory cell types, release of cytokine subsets. Start of immune and repair processes
     Intermediate (hours to several days)
15   Clot resolution, mobilisation of new subsets of different cell types as macrophages and fibroblast, release of new cytokine subsets, extracellulary protein matrix deposition, wound healing, adapted /recombinatory immune response, eventually a rejection process starts.

     Chronically stage
20   Scar formation processes (active for ever), memory formation of immune response
     Encapsulation

     The processes outlined above contribute to the above-discussed problems of having biosensors to function adequately *in vivo*. A useful review of implanted electrochemical
25   glucose sensors for the management of diabetes can be found in Heller et al., 1999.

     One State of the art manufacturer (Minimed Inc.) of biosensors has several patents, and of these drawings of sensor geometry and coatings can be found in e.g. US2001/0008931.

30   In the function of most glucose sensors based upon glucose oxidase, H_2O_2 is produced continuously. Hydrogen peroxide appears to be a ubiquitous molecule. Multiple papers have described high (usually $\geq 50\mu M$) levels of H_2O_2 as being cytotoxic to a wide range of animal, plant and bacterial cells in culture. However, levels of H_2O_2 at or below about 20-$50\mu M$ seem to have limited cytotoxicity to many cell types (Halliwell et al., 2000).
35
     WO94/10560 describes a glucose oxidase sensor with a catalase membrane which regenerates a part of the oxygen consumed by the glucose oxidation in order to improve the performance of the glucose sensor by making the regenerated oxygen available to the enzymatic reaction of the glucose oxidase.

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None of these references deal with the problem of the present invention which is to reduce H_2O_2 to a concentration much lower than the concentration where it will exert cytotoxic effects as described in detail in the following.

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DETAILED DESCRIPTION OF THE INVENTION

The present invention relates to glucose sensors producing reactive oxygen species . Examples of ROS producing glucose sensors are glucose oxidase based glucose sensors
10   which generate and release H_2O_2 to their surroundings when functioning.
In general terms such an electrochemical sensor according to the invention will comprise a working electrode which comprises the following:

1. glucose oxidase producing H_2O_2 and an H_2O_2 detecting electrode (central part)
15   2. diffusion compartment through which glucose and O_2 diffuse to the glucose oxidase and the electrode and excess H_2O_2 may diffuse out to the body
3. ROS removing compartment which may comprise catalase or other reactive oxygen species scavenger. It has a membrane function in that glucose, O_2 and other low molecular weight substances diffuse to the glucose oxidase and the electrode. Another important
20   function of the compartment is to avoid that excess H_2O_2 diffuses out to the body as described in detail in the following.
4. Semipermeable compartment which hinders access of cells and high molecular compounds to the central part but allowing access for molecules having a molecular weight of less than e.g. 500D.
25   5. Biocompatible compartment providing the interface between the sensor and the body having properties to avoid membrane biofouling

3 and 4 may be the same compartment, 4 and 5 may be the same compartment, and 3, 4 and 5 may be the same compartment.
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The primary object of the present invention is to provide means for assuring that the glucose sensor functions adequately. In the present context, the sensor functions adequately when there is a significant correlation between physiological relevant glucose concentrations and the signal from the sensor.
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The term 'monocyte chemotaxis' designates the processes by which a monocyte orients itself in a specific spatial relationship to a chemical stimulus. Monocyte chemotaxis may thus result in attraction and direction to the sites of various chemical substances.

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Biofouling has been described as the adhesion of proteins and other biological matter on the surfaces of a sensor and causing decreased sensor signal. Membrane biofouling is a process that starts immediately upon contact of the sensor with the body when cells,
5 proteins and other biological components adhere to the surface, and in some cases, impregnate the pores of the material. The membrane biofouling of the sensors outer membrane does impede analyte diffusion causing decreased sensor signal and it is believed that the adhering proteins are one of the main factors to modulate the longer term cellular and/or encapsulation process. Electrode fouling (electrode passivation) is a
10 process that occurs on the interior of the sensor when substances from the body are able to penetrate the outer membranes and alter the electrode surface and causing decreased sensor signal (Wisniewski et al., 2000).

In the present context biointerference is defined as the processes which disturb the sensor
15 signals executed around, on or in a sensor by the biological components of the body. The processes lead to altered diffusion conditions around the sensor caused by accumulation of cells or fouling of one or more, possibly all three types mentioned above.

The term 'encapsulation' is defined as an in vivo process in which fibroblasts, fibrocytes,
20 collagen, and giant cells provide adherent, impermeable, avascular barriers around or enclosing implants.

ROS (including H_2O_2, O_2^- and OH^-) are important chemical mediators in the regulation of signal transduction processes involved in cell growth and differentiation (Sauer et al.
25 2001). As example H_2O_2 induces activation of the interleukin-6 promoter activating nuclear factor-κB through NFκ-B inducing kinase (Zhang et al., 2001).

A first aspect of the present invention thus relates to a method of improving the performance of a ROS producing glucose sensor, said method comprising providing the
30 glucose sensor with a ROS removing compartment capable of reducing the diffusion of ROS out of the glucose sensor to a level at which biointerference is abolished or substantially reduced.

Another aspect of the invention relates to the use of a ROS removing compartment in a
35 ROS producing glucose sensor so that biointerference is substantially decreased or avoided. Yet another aspect of the invention relates to a ROS producing glucose sensor comprising a ROS removing compartment capable of reducing the diffusion of ROS out of the glucose sensor to a level at which biointerference is abolished or substantially reduced. Finally, one aspect of the invention relates to the use of such a sensor in a human. As

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stated above, examples of ROS producing glucose sensors are glucose oxidase based glucose sensors.

It is to be understood that the following description of features and embodiments of the
5 invention relates to all the above mentioned aspects of the invention.

TGFβ is a major local up-regulator of the extracellular matrix proteins in fibrosis. It also induces monocyte chemotaxis. TGFβ is activated by Reactive Oxygen Species (ROS). ROS are generated by reduction-oxidation reactions.
10

It is an object of the present invention to reduce the diffusion of ROSfrom a ROS producing glucose sensor, such as a glucose sensor based upon glucose oxidase, to a level where substantially no activation of TGFβ and substantially no monocyte chemotaxis occur. In preferred embodiments of the invention, the ROS is H_2O_2.
15

None of the prior art references have dealt with the problem of avoiding initiating the ROS cascade. In order to do to it, it is necessary to reduce the level of e.g. hydrogen peroxide to a level which is significantly lower than previously considered, i.e. to a level which is significantly lower than the level which has previous been considered safe, i.e. considerably lower than
20 20μM. In preferred embodiments of the invention the diffusion of ROS, such as H_2O_2, out of the sensor is reduced so that the concentration in the tissue surrounding the glucose sensor remains below 10μM.

The method of the invention may be accomplished by introducing in a ROS producing
25 glucose sensor, such as a glucose sensor based on glucose oxidase electrodes, a specially placed and specially composed compartment in the glucose sensor, which will minimise release of H_2O_2 and the related undesired tissue interaction and attraction of inflammatory cells. The compartment surrounds the electrode and may contain catalase and/or one or more other reactive oxygen species scavengers for removing ROS, such as hydrogen
30 peroxide, and their reactive oxidative decay products, and may be placed inside semipermeable and biocompatible outer compartments (see figure 1).

Placing catalase and/or one or more other reactive oxygen species scavengers in a semipermeable compartment placed between the ROS producing electrode compartment
35 and body tissue and making these compartments inaccessible for cells proteins and other higher molecular weight body substances and therefore minimise extensive oxidation damage reduces the accumulation of cells, fibrosis etc. and prolong the function of the sensor. By the method of the present invention the encapsulation process is substantially decreased which can be evidenced by the fact that the thickness of the fibrosis layer

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around the glucose measuring part of the sensor will be significant thinner when the sensor functions according to the method of the invention. Thus, in a histological section the thickness of the collagen capsule around the glucose measuring part of the sensor is less than 1 mm, such as less than 0,5 mm, preferably less than 0,1 mm, even more

5   preferably less than 0,05 mm, most preferably less than 0,01 mm after a functional period, which is several days, one week, several weeks, several months, such as 3 months, preferably 6 months, most preferably one year as described in the following.

In order to increase the function of the glucose sensor *in vivo* and avoid e.g. the undesired

10   calibration problems it is considered necessary to reduce the ROS, such as H_2O_2, to an amount, which is much lower than previously considered safe, i.e. considerably lower than $20\mu M$.

The present invention relates to use in a human of an implanted glucose oxidase based

15   glucose sensor of a ROS removing compartment comprising catalase and/or a reactive oxygen species scavenger in order to reduce the diffusion of ROS, including H_2O_2, out of the sensor to a level where biointerference is substantially decreased or avoided in spite of the fact that the sensor is implanted in the human for a prolonged period of time.

20   The present invention thus provides a sensor for which the necessary amount of calibration is reduced when compared to a similar glucose sensor without a ROS removing compartment. Thus, by use of the method of the invention an implanted device will only necessitate calibration no more than once a day, such as once every second day, once every third day, or even only once a week for a period of time which is several days, one

25   week, several weeks, several months, such as 3 months, preferably 6 months, most preferably one year.

In preferred embodiments of the invention the sensor is an implanted or semi-implanted sensor. Because of the decrease or avoidance of the biointerference, it is possible to have

30   the implanted sensor function adequately several months, such as 3 months, preferably 6 months, most preferably one year. By the term "semi-implanted" is meant a sensor which is partly implanted but wherein part of the sensor is present outside the body. In practical terms a such sensor can be placed and removed by the person himself without the aid of medical personal. An example of a such sensor is a needle sensor produced e.g. by

35   Minimed. By use of the method of the invention these sensors will function adequately for a long amount of time even if left within the body for at lest several days. Such semi-implanted sensors are thus in the present context within the concept of "implanted" sensors.

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The important issue is that the level of ROS in the ROS removing compartment is to be considerably lower than the level of ROS, such as H_2O_2 naturally present in the particular body compartment so that no positive concentration gradient for H_2O_2 towards the sensor exists.

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In preferred embodiments of the invention, the ROS removing compartment comprises catalase and/or one or more other reactive oxygen species scavengers. Examples of such reactive oxygen scavengers are polyphenols, such as as flavonoids, and plant phenolics, among them phenolic acids. The efficiency of phenolic compounds as anti-radicals and

10 antioxidants is diverse and depends on many factors, such as the number of hydroxyl groups bonded to the aromatic ring, the site of bonding and mutual position of hydroxyls in the aromatic ring. Other examples of reactive oxygen scavengers are natural phenolic antioxidants (alpha- hydroxytyrosol, tyrosol, caffeic acid, alpha-tocopherol) as well as commercial phenolic antioxidants (BHT and BHA) and carotenoids.

15

In preferred embodiments the level of ROS, especially H_2O_2 immediately outside the glucose sensor is below 5μM, such as below 3μM, e.g. below 2μM, preferably below 1μM, more preferably below 0.5μM, even more preferably below 0.3μM, most preferably below 0.2μM. In especially preferred embodiments the level of H_2O_2 immediately outside the

20 glucose sensor is below 0.1μM, such as below 0.05μM, e.g. below 0.03μM, preferably below 0.02μM, more preferably below 0.01μM, even more preferably below 0.001μM, most preferably substantially 0μM.

By use of the method according to the invention, the functional performance of the glucose

25 sensor *in vivo* is improved. In particular the necessary amount of calibration is reduced as the reduced biointerference resulting from the reduced level/gradient of ROS, such as hydrogen peroxide, will increase the stability of the sensor over time, thereby minimising the number of re-calibrations of the sensor necessary for adequate performance over prolonged time periods. Presently, it is necessary to calibrate the commercially available

30 sensors four times a day. By use of the method of the invention it is possible to prepare sensors which will only necessitate calibration no more than once a day, such as once every second day, once every third day, or even only once a week.

No such glucose sensors are presently available.

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LEGEND TO FIGURE

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The invention is illustrated schematically in the figure which shows schematically the working electrode of a glucose sensor comprising the following compartments:

1. glucose oxidase producing H_2O_2 and an H_2O_2 detecting electrode
5   2. diffusion compartment through which glucose and O_2 diffuse to the glucose oxidase and the electrode and excess H_2O_2 may diffuse out to the body
3. ROS removing compartment e.g. a catalase membrane
4. Semipermeable compartment which hinders access of cells and high molecular compounds to the internal part of the electrode.
10   5. Biocompatible compartment

EXAMPLES

Materials:

Electrochemical glucose needle sensors based on non-mediated glucose oxidase working
15   electrodes in which no catalytic outer membrane is present. The needle sensors may be of either the two-electrode type (e.g. as described by Wilson.G.S. et al in US 5,165,407) or three-electrode type.

The three- electrode type sensors are commercially available (e.g. MiniMed's continuous
20   glucose sensor available from Medtronic MiniMed, 18000 Devonshire Street, Northridge, CA 91325-1219, USA) or homemade (e.g. as described by Ege, H. in WO 89/07139).

The sensors are powered by a potentiostat/galvanostat. Potentiostats suitable for different sensortypes are commercially available (e.g. uAutolab type II from Eco Chemie B.V., P.O.
25   Box 85163, 3508 AD Utrecht, The Netherlands, or Amel instuments model 2059 from AMEL srl - Via S. Giovanni Battista de la Salle, 4, 20132 Milan – Italy).

Identical sensors are modified into two different groups C+ and C- by adding an extra outer membrane, where the sensors in the C+ group contains active hydrogen peroxide
30   degrading catalyst (e.g. catalase) and the C- does not (e.g. heat inactivated catalyst or placebo catalytic inactive substance e.g. albumin).The extra outer membrane may be made on basis of Polyurethanes, alginates or other biocompatible material and a final biocompatible outermost membrane may also be added if suitable for *in vivo* function.

35   Phosphate Buffered Saline (PBS), or other standard physiological buffer is needed for the *in vitro* measurement. Known amounts of glucose are added to buffer samples until glucose concentrations (1 mM - 30 mM range) relevant for *in vivo* measurements are reached. A small amount of preservative may be added (e.g. 1.2 mM sodium azide).

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The glucose-PBS samples are also used for the initial equilibration (or "priming") of the
sensors until a stable electric current measurement (at an applied working electrode
potential of 0.6 volt) is achieved (normally within about half an hour, if an initial potential
5 of about 1.1 volt for a few minutes is applied to the working electrode).

Hydrogen peroxide liberated from the C+ or C-sensors can be measured by different
commercially available peroxide test colour strip kits (MERCK EUROLAB A/S, Denmark) or
by titration methods known to the person of ordinary skill in the art of analytical
10 chemistry.

As the C+ or C- sensors are very small, the small amounts of hydrogen peroxide liberated
is detected electrochemically by a hydrogen peroxide sensor probe. This probe is the end
cross section of a thin (diameter 0.003"= 0.08 mm) Teflon coated platinum wire from A-M
15 Systems, Inc., PO Box 850, Carlsborg, WA 98324, USA , on which an electrode potential of
0.6 volt is applied relative to a reference electrode (e.g. Ag/AgCl homemade reference
electrode or available from CH Instruments, Inc., 3700 Tennison Hill Drive, Austin, TX
78738, USA).

20 A normal platinum wire (Goodfellow Cambridge Limited, Ermine Business Park,
HUNTINGDON, Cambridgeshire, PE29 6WR, England) is used as counter electrode and the
electric current between the probe electrode and the counter electrode is measured by an
potentiostat. As for the the glucose sensors an initial priming of the hydrogen peroxide
sensor probe is done at a little higher potential. Samples of Glucose–PBS with a further
25 addition of hydrogen peroxide to a hydrogen peroxide concentration in nanomolar to
millimolar range are used for this priming. Such buffer samples are also used for
establishment of calibration factors to be used in converting measured current to hydrogen
peroxide concentration.

30 For some *in vivo* histological studies identical sensors are modified into two other groups
H- and H+ by heat inactivation (20 -180 sec in room temperate water (H-group) or boiling
water (H+group)) without adding any further membrane.

Control of the heat inactivation of the hydrogen peroxide producing glucose oxidase in the
35 H- group is done *in vitro* using the potentiostat. It is controlled that the H- does not
respond to changes in glucose concentration , but still responds to changes in hydrogen
peroxide concentrations. For this suitable samples of Glucose-PBS buffer with or without
hydrogen peroxide (0,05-2 mM range) are used.

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For some further *in vivo* histological studies of local effects of subcutaneous infusion of
very small amounts of hydrogen peroxide, two separate pumps P- and P+ (type H-TRON
plus V100 with connected infusion set Tender PT17/110 II from Disetronic Medical
Systems, Inc., USA) are used. The P- pump is filled with standard physiological buffer with
5   addition of hydrogen peroxide (less than 4% by volume). The P+ pump is filled with
standard physiological buffer without addition of hydrogen peroxide. The pumps are then
identically programmed to deliver over a few days very few micro liters for every 3-10
minutes.

10

Experiment 1

In vitro characterisation:

15 A.   The C+ and the C- sensors are connected to the potentiostats and equilibrated in PBS,
to which a known amount of glucose has been added relevant for *in vivo*
measurements. The time for reaching the initial stable current is noted (normally
within half an hour) and also the response times to reach new stable plateau's of
currents corresponding to various glucose concentrations are noted. Also and most
20   important the differences in hydrogen peroxide liberated from the sensors into the
buffer is detected. This can be done by measuring the hydrogen peroxide concentration
gradients formed from the surface of the sensor out in the buffer. The gradients
formed are detected by changing the distance between glucose sensor surface and the
electrochemical hydrogen peroxide probe. This is done with either the glucose sensor
25   or electrochemical hydrogen peroxide probe fixed to a measure table or
micromanipulator with a micrometer scale. The distance of the probe from the sensor
surface is incrementally reduced or increased and recorded together with the
corresponding levels of measured current of the hydrogen peroxide probe.
     B.   Differences in hydrogen peroxide liberated from the sensors can also be measured in
30   samples of glucose-PBS buffer after the glucose sensors has worked overnight in the
buffer (preferably with a glucose concentration higher than 10 mM) . This can be done
by different commercially available peroxide test colour strip kits or by titration as
described above.
     C.   An *in vitro* cell assay (e.g. as described in Callahan et al., 1990) using amount of killed
35   cells due to liberation of hydrogen peroxide from the different groups of sensors can
also be used in the characterisation.

Experiment 2

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After the difference is established *in vitro* between C+ and C- group sensors, the same sensors are tested for differences *in vivo*.

5 Suitable laboratory animals are pigs or dogs.
A. To show the C+group sensors advantages over the C- group, the sensors are implanted and the current is measured over some days (e.g. three days) together with blood sampling at some fixed time points (e.g. morning and evening). The blood samples are analysed for glucose concentration with standard methods (e.g. test strips
10   and glucose meter in InDuo available from Novo Nordisk A/S, Denmark, or by use of laboratory instruments well known in standard clinical chemistry departments). From this the sensors' performance are evaluated (precision, interval needed for calibration and lifetime). These performance characteristics may be supplemented with histological analysis of the resulting tissue around the implanted sensors with special
15   emphasis on signs of killed cells and total amount of cells attracted to the sensors as well as signs of fibrosis, such as presence of collagen capsule around the glucose measuring part of the sensor.
B. To further support the histological analysis of experiment 2A the H- and H+ sensor group are also implanted *in vivo* for some days. At the end of the experiment,
20   histological analysis of the tissue around the implanted sensors are conducted with special emphasis on signs of killed cells and total amount of cells attracted to the sensors as well as signs of fibrosis, such as presence of collagen capsule around the glucose measuring part of the sensor .
C. Also, to further support the histological analysis of experiment 2A, one animal is
25   infused subcutaneously for a few days *in vivo* using both the P- and P+ pumps. Subsequently, the tissue at the site of infusion is analysed with special emphasis on signs of killed cells and total amount of cells attracted to the infusion sites as well as signs of fibrosis.

30 The extent of fibrosis can be evaluated using a standard techniques. The most common staining technique is known as Hematoxylin and Eosin (or H&E) staining. In order to stain the sections the wax needs to be removed. This is done using a wax solvent such as xylene. The slide is then hydrated using a series of descending alcohols (100%, 95%, 70%) and then water. The
35 slide is then immersed in Hematoxylin stain, rinsed in running water (preferably alkaline), followed by staining with Eosin, and rinsing in water.

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As an alternative to using H&E staining, the presence of collagen fibres can be determined using methods of histological staining known to a person with skills in the art. Examples of such stainings are the Van Giesen staining and the Masson Trichrome staining.

5   From the experiments it is clear that for optimal performance it is not enough to keep the amount of hydrogen peroxide liberated to the body lower than the level where cytotoxic (cell killing) effects are seen. In order to prevent cell attracting around the sensors, the concentration of the hydrogen peroxide hydrogen peroxide liberated to the body must be as low, or lower, as the concentration seen in the C+ group sensors.

10   **REFERENCES**

Callahan, H. L.; Crouch, R. K.; James, E. R. Hydrogen Peroxide Is the Most Toxic Oxygen Species for Onchocerca Cervicalis Microfilariae. *Parasitology* **1990**, *100 Pt 3,* 407-415.

Halliwell, B.; Clement, M.; Long, L. Hydrogen peroxide in the human body. *FEBS Letters*
15   **2000**, 486, 10-13

Heller, A. Implanted Electrochemical Glucose Sensors for the Management of Diabetes. *Annu. Rev. Biomed. Eng.* **1999** 01, 153-175

20   Jobst, G.; Moser, I.; Varahram, M.; Svasek, P.; Aschauer, E.; Trajanoski, Z.; Wach, P.; Kotanko, P.; Skrabal, F.; Urban, G. Thin-Film Microbiosensors for Glucose-Lactate Monitoring. *Analytical Chemistry* **1996**, 68, 18, 3173-3179

Sauer, H.; Wartenberg, M.; Hescheler, J. Reactive Oxygen Species As Intracellular
25   Messengers During Cell Growth and Differentiation. *Cellular Physiology and Biochemistry* **2001**, 11, 173-186

Urban, G.; Jobst., G.; Keplinger, F.; Aschauer, E.; Fasching, R.; Svasek, P. Miniturized integrated biosensors. *Technology and Health Care*, 1994, 1, 215-218
30
Urban, G.; Jobst, G.; Aschauer, E.; Tilado, O.; Svasek, P.; Varahram, M.; Ritter, Ch.; Riegebauer, J. Performance of integrated glucose and lactate thin-film microbiosensors for clinical analysers. *Sensors and Actuators B,* **1994**, 18-19, 592-596

35   John Wiley & Son, "Biosensors in the body", 1997, page 210

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Zhang, J.; Johnston, G.; Stebler, B.; Keller, E.T. Hydrogen Peroxide Activates NF Kappa B and the Interleukin-6 Promoter Through NF Kappa B-Inducing Kinase. *Antioxidants & Redox Signaling* **2001**, 3, 493-504

5 US2001/0008931
   US 5,165,407
   WO 89/07139
   WO94/10560